

Real-time imaging of suction blistering in human skin using optical coherence tomography

Joana C.O. Carvalho,¹ Jonathan A. Palero,^{2,*} and Martin Jurna²

¹Faculty of Sciences, University of Lisbon, Campo Grande, Portugal

²Philips Research - Personal Care & Wellness, High Tech Campus 34, 5656 AE Eindhoven, The Netherlands

*Jonathan.Palero@Philips.com

Abstract: Separation of skin epidermis from the dermis by suction blistering has been used with high success rate for autologous skin epidermal grafting in burns, chronic wounds and vitiligo transplantation treatment. Although commercial products that achieve epidermal grafting by suction blistering are presently available, there is still limited knowledge and understanding on the dynamic process of epidermal-dermal separation during suction blistering. In this report we integrated a suction system to an Optical Coherence Tomography (OCT) which allowed for the first time, real-time imaging of the suction blistering process in human skin. We describe in this report the evolution of a suction blister where the growth is modeled with a Boltzmann sigmoid function. We further investigated the relationship between onset and steady-state blister times, blister growth rate, applied suction pressure and applied local skin temperature. Our results show that while the blister time is inversely proportional to the applied suction pressure, the relationship between the blister time and the applied temperature is described by an exponential decay.

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1. Introduction

Suction blistering is a technique used for autologous skin epidermal grafting in burns, chronic wounds [1] and vitiligo transplantation treatment [2]. Notably, suction blistering presents minimal patient discomfort and alleviated need for anesthesia during the procedure since the cleaving occurs in the basal membrane where the pain sensory organs are sparsely present. First reported in 1978 by Unna [3] and extensively studied by Kiistala and Mustakallio [4], epidermal grafting has yet to gain wide acceptance for dermatological and dermatosurgical applications largely because harvesting techniques have been tedious and time consuming.

Under normal circumstances, the molecular interactions of the structural proteins of the epidermis, the dermal-epidermal junction or DEJ, and the dermis sustain the scaffolding of the skin. Blisters can occur either by protease degradation of the structural protein or by alteration of the protein-protein interaction or adhesive function of the molecules. Blister formation comprises three processes which occur successively and simultaneously [5]: 1) loosening of the structure due to diminished cohesion of epidermal cells or a weakened DEJ; 2) discontinuity, which involves cleft formation within keratinocytes or at different levels of the junction zone, and; 3) fluid accumulation, which involves movement of fluid into the damaged tissue region. The dynamic physical changes involved in the formation of blister during suction blistering has not been quantified until now.

Several studies have attempted to elucidate the dependence of the blistering time to different factors including, size of the blistering aperture, applied suction pressure and local skin temperature [4, 6]. For instance, it has been shown that the product of suction pressure and blistering time is constant, which suggest a viscous nature of DEJ [10]. On the other hand the adherence decreased exponentially with the increase of skin temperature. It is however difficult to make a comparison between these studies since the blistering time are all based on subjective assessment by visual inspection.

The present study aims to provide a better understanding on the dynamics of epidermal-dermal separation particularly on the onset time, steady-state time, and rate of suction blister formation. We also provide an objective definition of these parameters based on analysis of real-time Optical Coherence Tomography (OCT) images captured during suction blistering. To the best of our knowledge, this study reports for the first time real-time tomographic imaging of the epidermal-dermal separation during suction blistering in human skin. Finally, we establish the dependence of the onset and steady-state time of suction blistering to the suction pressure and applied temperature.

2. Materials and methods

2.1. Sample preparation

Freshly excised human abdominal skin tissue samples were obtained with consent from the donors. The skin samples were stored at -20°C until the time of suction blister treatment.

2.2 OCT integrated with suction system

The experimental setup (see Fig. 1) comprised a home-built suction chamber coupled to the imaging probe of the OCT system Callisto (Thorlabs GmbH, Lübeck, Germany). During suction blistering, the skin domes to a height that exceeds the field of view of OCT. We compensated for this problem in the design of the coupler by integrating a height adjustment ring. Another challenge in the development of such a coupling device is in the design of the suction chamber, wherein obstruction of the optical path of the OCT beam is inevitable. In our design, we integrated in the interface between the OCT system and the suction chamber an anti-reflection optical window (WG10530-B - Ø1/2" N-BK7 Broadband Precision Window, AR Coated: 650 - 1050 nm, $t = 3$ mm, Thorlabs) that allows transmission of light with minimal distortion and minimal intensity loss ($<1\%$). The suction chamber was also designed to accommodate a removable PMMA plate interface to the skin providing flexibility in

suction blistering with different circular opening diameters. In the report, we described results using a circular opening diameter of 1.5 mm. We further designed and constructed aluminum plate interfaces compatible with the OCT-suction coupler that allow us to apply heat into the skin tissue in a well-controlled manner using a thermocouple. During experiments, the pressure was monitored and regulated by conventional pressure gauges and valves.

2.4 Acquisition and analysis of OCT images

OCT images were acquired every ~ 2 s during each suction blistering experiment using a field of view of 3.0 mm (x-direction) \times 1.2 mm (z-direction) with associated pixel dimension of 1244×490 . Typical OCT images are shown in Fig. 2. During suction blistering, the skin is observed to initially form a dome within the plate opening and subsequently a single dark region or multiple regions are formed between the epidermis and the dermis. The dark region was observed to grow in time. Analysis of the OCT images after acquisition, particularly on the evolution in the dark region area, allowed us to obtain information on the qualitative dynamic formation of blisters and the associated quantitative blister growth during suction blistering.

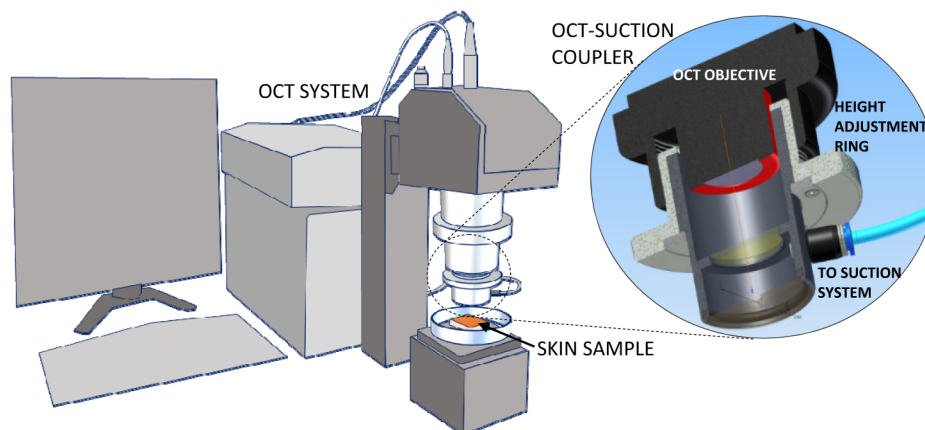


Fig. 1. Experimental setup showing the OCT system, home-built OCT-suction coupler and the skin sample.

3. Results

Previous histological studies have shown that skin suction blistering results in the separation of the epidermis and the dermis. In this study, we monitored the growth of the blister area during suction blistering as exemplified by the OCT images shown in Fig. 2. Here, suction pressure of 600 mmHg was applied to the skin through a circular suction orifice with a diameter of 1.5 mm. The measured skin tissue temperature during suction blistering was 21°C.

Three phases of blister formation during suction blistering were qualitatively identified: skin doming phase, growth phase and steady-state phase. The initial phase is characterized by doming of the skin due to the suction pressure acting on the viscoelastic skin tissue (see Fig. 2(A) to 2(C)). While the skin bulk structure is gradually modified and an increase in the size of the skin dome is observed, the epidermal and dermal layers remain attached to each other. At a critical time, formation of a dark region or regions between the epidermis and the dermis is observed (Fig. 2(D)). This is the onset of the growth phase where the blister grows in size (Fig. 2(D) to 2(F)). Here, the initial microscopic blisters or clefts can be initiated in one or several locations, not necessarily simultaneously (for example, see Fig. 3). We hypothesize that during the growth phase, the integrity of the epidermal-dermal junction (EDJ) is compromised by the force exerted by the dermal interstitial fluid to the epidermal layer.

Moreover, the rate of EDJ integrity degradation increases with the surface area of the separation, therefore a nonlinear rapid growth in blister size occurs during this phase. As the epidermal layer tension increases with the increase in blister area, the blister growth slows down until an equilibrium state is reached. In the equilibrium or steady-state phase, modification to the skin structure ceases (Fig. 2(G), 2(H)).

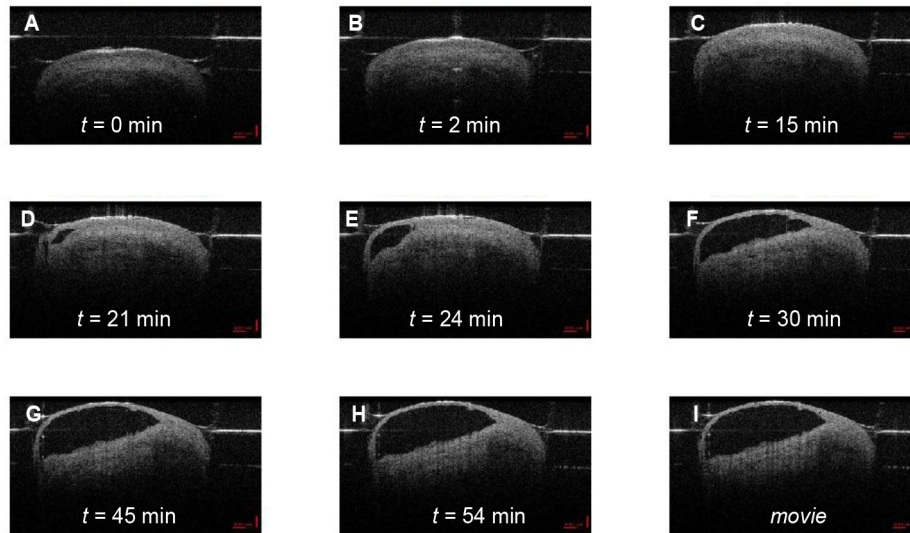


Fig. 2. Different phases of the blister formation process. During the first 15 minutes skin doming was observed (A to C). At 21 minutes the first cleft is observed (D) which grew in size (D to F). A steady-state phase is achieved at 45 minutes (G). A movie of the blister formation is shown in I with a speed factor of $\sim 1000 \times$ (see Visualization 1).

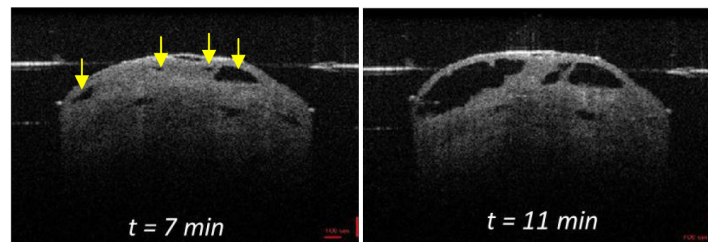


Fig. 3. Initiation of microblisters from multiple sites (yellow arrows) and eventual mergence at 7 min and 11 min, respectively.

To gain a deeper understanding on the dynamics of suction blister formation and the dependency to the applied suction pressure and tissue temperature, we quantified the time-dependent blister area from the OCT images. The data were then fitted with Boltzmann sigmoid function and from the fit we obtained the onset and steady-state blister times. Here, we define the onset blister time, t_{10} and the steady-state blister time, t_{90} as the time points when the normalized blister areas are 0.1 and 0.9, respectively. These two time points, t_{10} and t_{90} , can be used to quantitatively define the three phases of blister formation as described above.

Shown in Fig. 4(A) are the normalized blister area time profiles during application of different suction pressures: 150 mmHg, 300 mmHg, 450 mmHg and 600 mmHg. The skin temperature was measure to be $\sim 21^\circ\text{C}$. Here we observed that blister time profiles are characterized by “jumps” (see for example data regions indicated by arrows in Fig. 4(A)) which we attribute to non-simultaneous initiation of microblisters from multiple sites also observed in the movie data. Shown also in the figure are the Boltzmann sigmoid function fits,

showing good agreement with the data. It is evident from the plot that higher applied suction pressure results in shorter delay in both the onset and steady-state blister times. Plotting the blister times, t_{10} and t_{90} , against the suction pressure, we observed an inverse relationship between blister time and suction pressure (see Fig. 4(B)). This is in agreement with previously reported theoretical analysis on suction blister formation based on the viscous slip model [4]. The fitted function: $t_{10/90} = t_0 + A_{10/90} \cdot p^{-1}$, where p is the suction pressure in mmHg and t_0 and $A_{10/90}$ are fitted constants, resulted in $t_0 = -50$ min, $A_{10} = 2.7 \times 10^4$ mmHg·min, $A_{90} = 3.8 \times 10^4$ mmHg·min. Based also on Fig. 4(B), we observe an exponential growth dependence between the blister growth rate and the suction pressure with the fit function defined as $r_p = B \cdot e^{k_p \cdot p}$, where r_p is the blister growth rate in %·min⁻¹, p is the suction pressure in mmHg and B and k are fitted constants. Here, we obtained: $B = 0.63\%$ ·min⁻¹ and the exponential growth constant; $k_p = 4.3 \times 10^{-3}$ mmHg⁻¹.

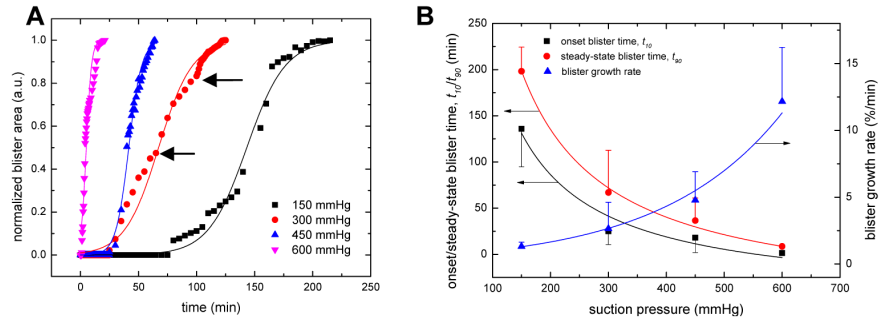


Fig. 4. A: Typical blister growth curves showing normalized blister area evolution for different pressures at ~21°C. B: Dependence of the onset and steady-state blister times, t_{10} (black squares) and t_{90} (red circles), respectively, on the suction pressure at ~21°C. The blister growth rate (blue triangles) shows good fit with an exponential function (blue line) against the suction pressure. For clarity, only positive or negative error bars are shown for the data.

As shown in Fig. 5(A), an increase in skin temperature, from 21°C to 39°C, shortens the delay to both the onset and steady-state blister times. The relationship between the onset and steady-state blister times and the applied temperature is found to be an exponential decay (see Fig. 5(B)) consistent with previous reports [5]. The exponential fitting function is described by; $t_{10/90} = C_{10/90} \cdot e^{-\theta_{10/90} \cdot T}$, where T is the applied temperature in °C and $C_{10/90}$ and k_T are fitted constants. Here, we obtained: $C_{10} = 76.7$ min; $C_{90} = 116.4$ min; $\theta_{10} = 0.08$ °C⁻¹, and; $\theta_{90} = 0.11$ °C⁻¹.

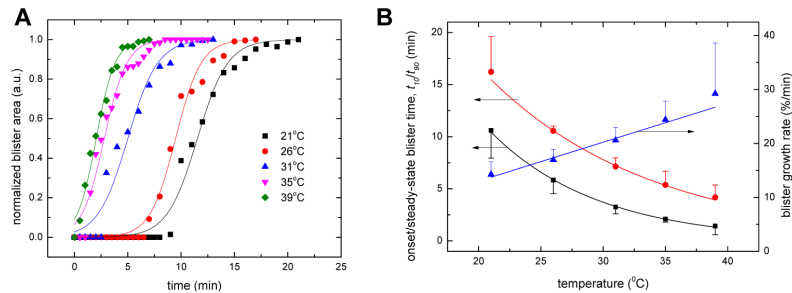


Fig. 5. A: Typical blister growth curves showing normalized blister area evolution for different temperatures using a suction pressure of 600 mmHg. B: Dependence of the onset and steady-state blister times, t_{10} (black squares) and t_{90} (red circles), respectively, on the local skin temperature using a suction pressure of 600 mmHg. The blister times fit with exponential decay curves (black and red lines). The blister growth rate (blue triangles) shows positive linear relationship with skin temperature.

The blister growth rate is observed to increase linearly with the applied temperature range (see Fig. 5(B), *blue triangles*) described by the linear fitting function: $r_T = r_0 + D \cdot T$, where r_T is the blister growth rate in $\% \cdot \text{min}^{-1}$, T is the applied temperature in $^{\circ}\text{C}$, and r_0 and D are the fitted constants. Here we obtained $r_0 = -1.39\% \cdot \text{min}^{-1}$ and $D = 0.72\% \cdot \text{min}^{-1} \cdot ^{\circ}\text{C}^{-1}$. The effect of increase in temperature to the blister time may be attributed to the increase in viscosity of the interstitial fluid [4], changes in the cells of the basal layer of the epidermis [6], alteration of the permeability of the keratinocytes of the basal layer leading to increased susceptibility to the force exerted by the interstitial fluid, and possibly thermal denaturation and detachment of the hemidesmosomes from the basement membrane [9].

In summary our results show that the onset and steady-state blister times can be reduced by an increase in applied suction pressure or increase in temperature. We observe agreement of our results with previous reports [6, 7, 10] regarding the dependency of the blister times with applied suction pressure and temperature. Finally it is noteworthy to mention that for the first time a potentially robust and consistent method that quantitatively defines blister times is described in this study.

4. Conclusion

To the best of our knowledge, this work reports for the first time real-time tomographic imaging of epidermal-dermal separation using Optical Coherence Tomography (OCT) during suction blistering of human skin. We observed three phases during suction blistering: skin doming phase; growth phase, and; steady-state phase. We quantitatively modeled this evolution of a suction blister with a Boltzmann sigmoid function which allowed us to elucidate the relationship between onset and steady-state blister times, blister growth rate, applied suction pressure and applied local skin temperature. Our results show that while the blister time is inversely proportional to the applied suction pressure, the relationship between the blister time and the applied temperature is described by an exponential decay. Moreover, the blister growth rate is found to be exponentially related to the applied pressure and linearly related to the applied temperature. The results particularly on the qualitative dependence of the blister growth rate with the pressure and temperature presented in this ex vivo study may very well translate to the in vivo case. However, we estimate that due to the better adherence of the viable epidermis to the dermis in the in vivo skin, both the onset and steady-state blister times are substantially longer for the in vivo skin. Although the present study focused only on the effects of applied pressure and temperature on suction blister dynamics, in vivo studies may include other factors such as age that may affect the blister dynamics. In a recent systematic review and meta-analysis, it was concluded that temperature and age are the strongest predictors of suction blistering time [11]. Overall, this study allows further development and improvement of systems and methods that rely on precise and well-defined skin blister formation from autologous skin epidermal grafting in burns, chronic wounds and vitiligo transplantation treatment to interstitial fluid extraction.